Electrochemical Properties of Model Cornpounds of Nucleic Heterocycles in Aqueous Solutions

Daniel Thevenot, Gilbert Hammouya

Reprinted from « Biological Aspects of Electrochemistry »
Experientia Suppl. **18**, 647-665 (1971)

Electrochemical Properties of Model Compounds of Nucleic Heterocycles in Aqueous Solutions

by D. THÉVENOT and G. HAMMOUYA
Laboratoire d'Energétique Electrochimique, 10 rue Vauquelin, Université Paris VI, 75-Paris 5, France

Summary

Taking into account the biological significance of electron-exchange properties of nucleic heterocycles, we have studied the electrochemical reduction of their simplest model compound *i.e.* the pyrimidine, using drop-time controlled polarography and cyclic voltammetry on mercury drop and stationary vitreous carbon electrode. The characteristics of the different reduction waves have been studied with respect to the different experimental parameters: pH, nature and concentration of buffer solutions, pyrimidine concentration, mercury flow and mechanically controlled drop-time, temperature, nature of the electrode, etc. The analysis of the experimental data is discussed in terms of energetical significance of the phenomena observed. Evidence is given of a chemical association step, possibly a dimerization, occuring between the two reduction steps in acid media.

In contrast with pyridine, flavin and porphyrin heterocycles, nucleic heterocycles *i.e.* pyrimidine and purine derivatives are rarely considered to have electron-exchange properties of biological significance. This is in contradiction to several experimental findings of pyrimidines and purines directly participating in biological electron transfer reactions. According to SKULACHEV [1] adenosine diphosphate (ADP) plays the role of a hydrogen carrier in the respiratory chain, the purine ring being reduced into 1,6 dihydropurine. Similarly, BARLTROP [2] presented chemical evidence of a mechanism involving the adenine ring of nicotinamide adenine dinucleotide (in its oxidized NAD^+ or reduced NADH form) in the oxidation of NADH by flavoprotein. Pyrimidines and purines are also known to form charge-transfer complexes with several electron acceptors [3–6]: this complex formation is strongly dependent on the electrochemical properties of the compounds and is frequently used to explain biological reactions.[7]

The pyrimidine and purine derivatives of biological significance (Figs. 1 and 2) contain the pyrimidine ring on which the reduction site is situated. Since several studies [8,9] were carried out on the influence of the substituent position on the reduction properties, it is generally agreed that the first reduction steps of these compounds are identical *i.e.* the reduction of the 3,4 double bound of pyrimidines and the 1,6 double bound of purines.

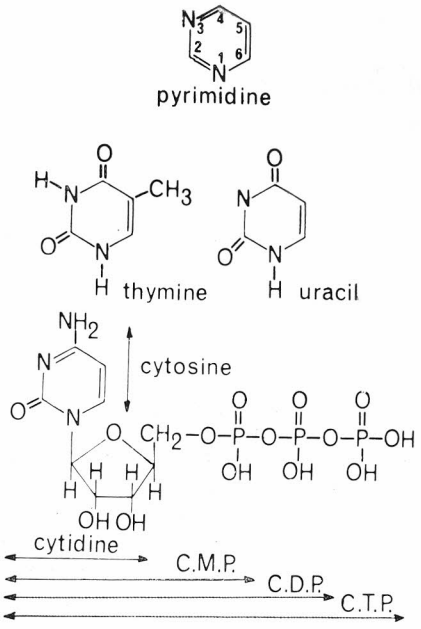


Fig. 1.
Formulas of pyrimidine derivatives.

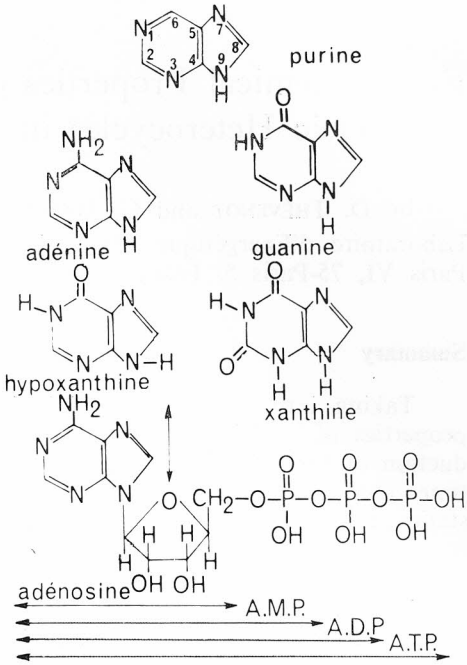


Fig. 2.
Formulas of purine derivatives.

Thus the pyrimidine itself is used as the simplest model compound of nucleic heterocycles: it has been studied in the largest pH range (Tab. 1) and presents one of the less negative reduction potential (Tabs. 2 and 3).

We have focused our work on the pyrimidine electrochemical behavior on different electrodes. Conventional polarography at a dropping mercury electrode has been studied by several research workers[10] the most detailed investigation being that of SMITH and ELVING.[8,11,12] *a.c.* polarography and cyclic voltammetry at a hanging mercury drop electrode and at a pyrolitic graphite electrode have been studied more recently by O'REILLY[13] and DRYHURST.[14] There is general agreement between the results achieved by *d.c.* polarography and the other electrochemical methods giving five reduction waves appearing over the pH-range 0.5–13 (Fig. 3). In strongly acidic media, a pH-dependent 1e wave I is shown. At about pH 3, a pH-independent 1e wave II emerges from the back-ground discharge. These two waves merge near pH 5 to form a pH-dependent 2e wave III. Near pH 7, a pH-independent 2e wave IV emerges from the background and at pH 9, it merges with wave III to form the pH-dependent 4e wave V. The following mechanisms (presented in Fig. 4) have been postulated: wave I is a 1e reduction of pyrimidine to a neutral radical; wave II is a 1e reduction of the latter to a dihydropyrimidine while wave III is a composite of these two steps; wave IV is the 2e reduction of the dihydrospecies to a tetra-

Table 1. pH range used in electrochemical study of pyrimidine and purine derivatives: reduction shown as possible (——) and impossible (– – –), oxidation presented as possible (═══) and impossible. (= = =) (□) pK_a values of these compounds in aqueous solution at room temperature. [32–39]

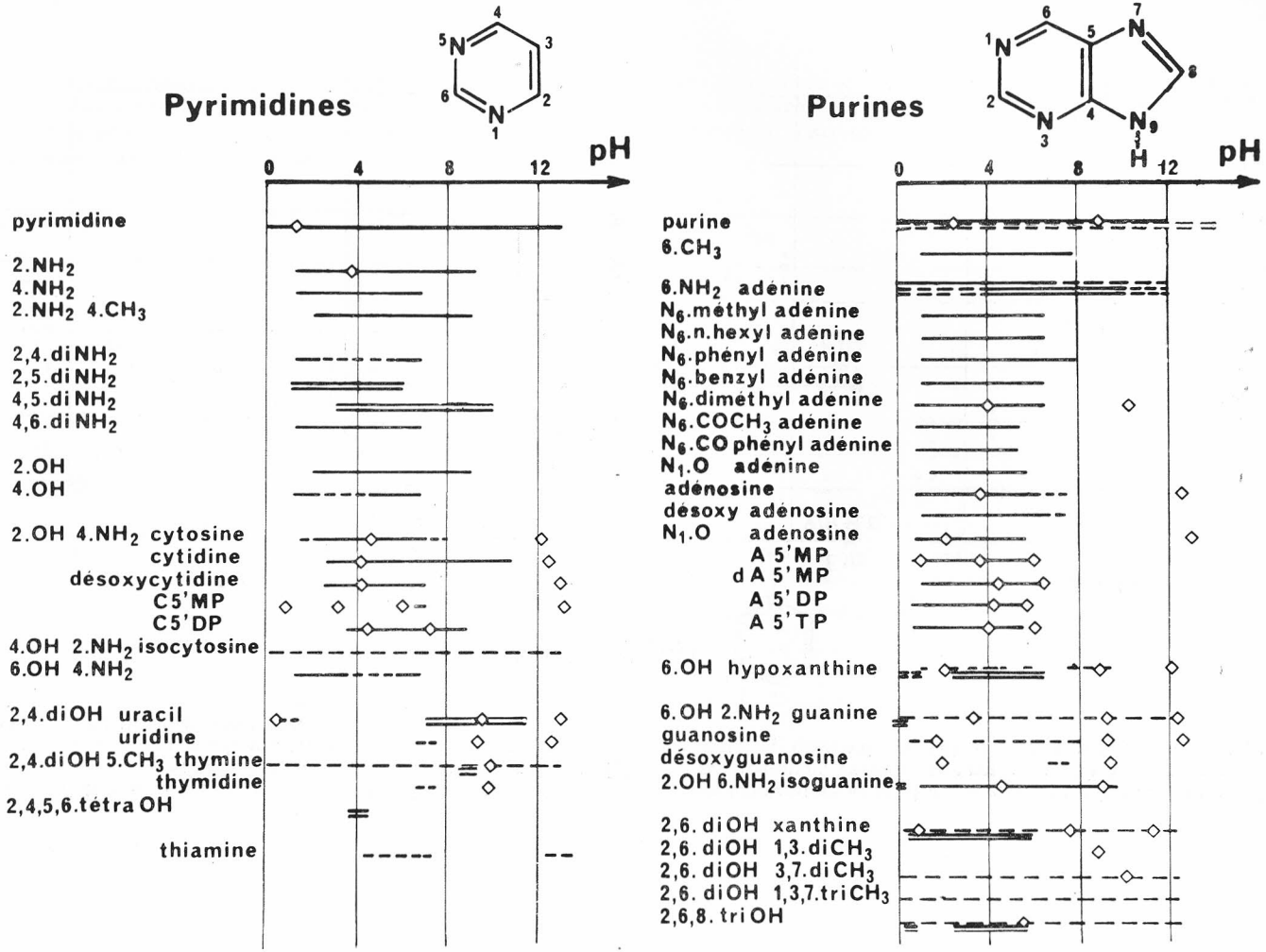


Table 2. Potential values of pyrimidine derivatives in aqueous solution at room temperature at pH 5.0, 7.0 and 9.0: effect of nature and position of the simplest substituents. Reduction is observed by (□) *d.c.* polarography, cyclic voltammetry on (◇) hanging mercury drop or (○) pyrolitic graphite electrode, and (Δ) *a.c.* polarography. Oxidation is observed on (■) *d.c.* polarography and cyclic voltammetry on platinum electrode.

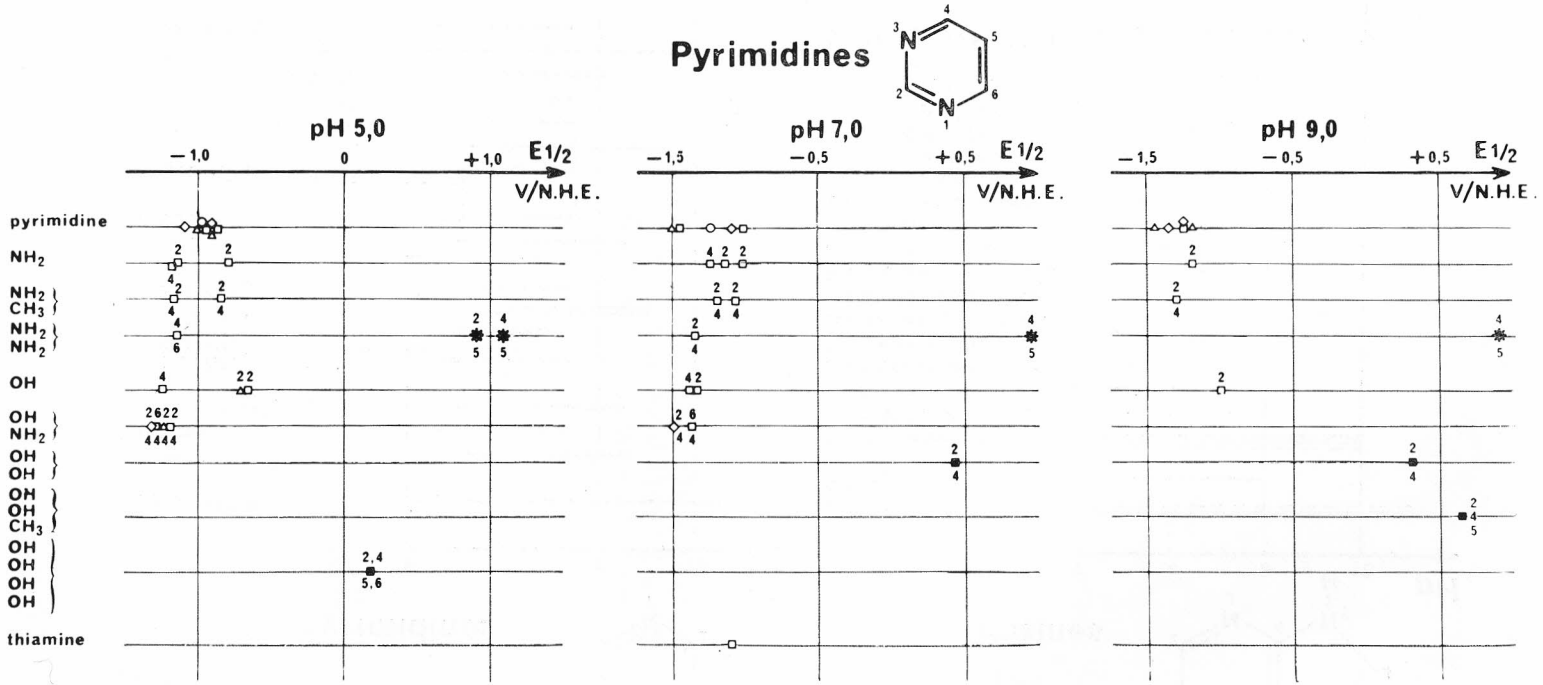
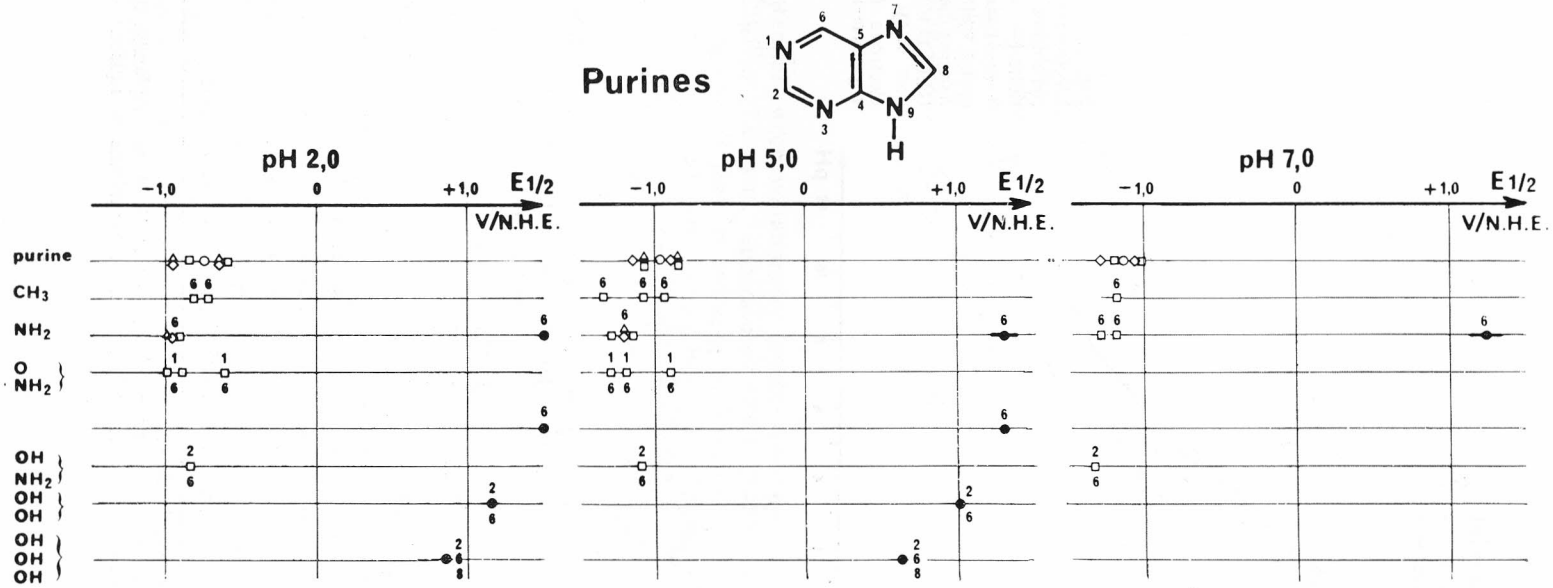


Table 3. Potential values of purines derivatives in aqueous solution at room temperature at pH 2.0, 5.0 and 7.0: effect of nature and position of the simplest substituents. Reduction is observed by (□) *d.c.* polarography, cyclic voltammetry on (◇) hanging mercury drop or (○) pyrolitic graphite electrode, and (Δ) *a.c.* polarography. Oxidation is observed on cyclic voltammetry on (⊷) hanging mercury drop, (●) pyrolitic graphite.



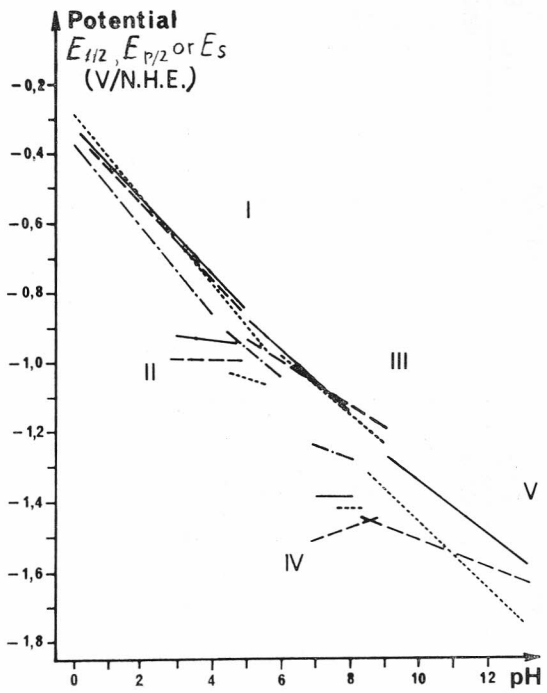
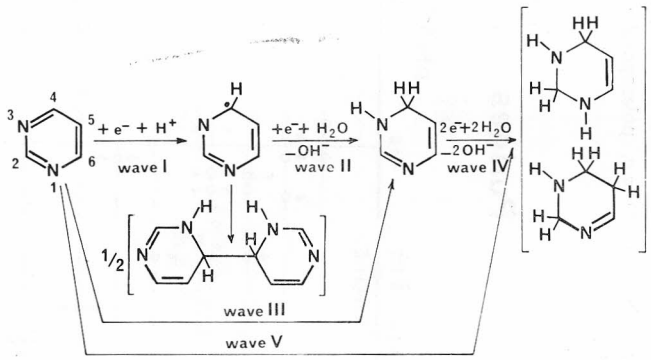


Fig. 3.
Electrochemical behaviour of pyrimidine in aqueous solution: (——) *d.c.* polarography, (— —) *a.c.* polarography and cyclic voltammetry on (.....) hanging mercury drop electrode (H.M.D.E.) 0,026 V s^{-1} and (· – · – ·) pyrolitic graphite electrode (P.G.E.) 0.060 V s^{-1} [8,10–14]



Waves	pH	Concentration (mole. l^{-1})	$E_{1/2}$ (mV/ N.H.E.)	$I = \frac{-I_d}{C.m^{2/3}.t^{1/6}}$	$\lvert E_{3/4} - E_{1/4} \rvert$ (mV)	number of e^- (coulometry)
I	0.5 to 5	0.3 to $4 \cdot 10^{-3}$	– 334 – 105 pH	2.0 to 2.5	44 ± 7	0.94 ± 0.01
II	3 to 5	0.3 to $0.4 \cdot 10^{-3}$	– 900 – 11 pH	2.2 to 2.7	95	1.08 ± 0.06
III	5 to 8	0.3 to $5 \cdot 10^{-3}$	– 438 – 89 pH	4.3 to 4.7	41 to 95	2.0 ± 0.3
IV	7 to 8	$0.3 \cdot 10^{-3}$	– 1358 – 5 pH	4,8		
V	9 to 13	0.4 to $1.5 \cdot 10^{-3}$	– 563 – 79 pH	8 ∽ 9 (6 at pH 13)		3.93 ± 0.06

Fig. 4 and Table 4.
Mechanisms proposed by ELVING *et al.*[8,11,12] for explaning the *d.c.* polarographic and coulometric behaviour of pyrimidine in aqueous solution.

hydropyrimidine while wave V is a composite of waves III and IV. The relevant numerical data are shown in Tab. 4.

Past papers [15–18] have shown that the above suggested mechanisms do not agree with the *d.c.* polarography behaviour of pyrimidine when its concentration is higher than a few millimoles per litre. Moreover, we are thinking that further investigations are necessary to prove the dimerization of the radical produced by wave I and to explain pH-independent wave II.

Experimental

Reagents

Buffer solutions were prepared from chemicals of analytical reagent grade at a concentration at least 50 times greater than those of pyrimidine (usually 0.5 *M* acidic and 0.5 *M* basic form). Following buffers were used: chloride, oxalate, phosphate (first and second pK_a), citrate (first, second and third pK_a), phtalate (second pK_a), imidazole, tris, borate (first and second pK_a), carbonate (second pK_a) and potassium hydroxide.

Pyrimidine (SCHUCHARDT and FLUKA A.G.) was *purum* grade; its polarographic pattern gave no evidence of any electroactive impurity.

Apparatus

Triangular potential sweep voltammetry (cyclic voltammetry) was carried out with different TACUSSEL apparatuses (PRT 20-2X, GSTP, two ADTP) and a TEKTRONIX R 564 B storage oscilloscope.

Direct current polarography was carried out with different TACUSSEL apparatuses (PRT 500 LC, Servovit 9 B, S6RZ) and a SEFRAM XY bigalvanometric recorder.

A conventional thermostated three electrode cell was used. The D.M.E. was synchronized with the potential sweep by a TACUSSEL electrical hammer (MPO animated by a GCMR). For *d.c.* polarography the drop-time was regulated at 0.5 s, thus eliminating the erratic variations of drop-time at very negative potentials or the use of Desicote or related products.

General voltammetric procedures

Test solutions were prepared by adding small quantities of highly concentrated pyrimidine solutions (0.5 and 0.05 *M*) to a known volume of buffer. Solutions were regularly deoxygenated for at least 15 min before examination and were kept under a nitrogen atmosphere.

For *d.c.* polarography, the potential was allowed to change at a rate of 0.25 V/min, the highest sweep rate allowed by the XY recorder used.

Although an Ag|AgCl, saturated KCl reference electrode was used, all potentials cited are referred to the N.H.E. at 25.0 $\pm$ 0.2 °C, temperature of the thermostated cell.

Results

Polarographic behavior of pyrimidine in very dilute aqueous solutions

Over the pH-range 0.3-9.7 pyrimidine in very dilute aqueous solution (10^{-4} *M*) gives four polarographic waves (Fig. 5). At low pH, a single pH-dependent wave is observed (Wave I). At about pH 2.0, a pH-independent wave II, whose current is much more than that of wave I, emerges

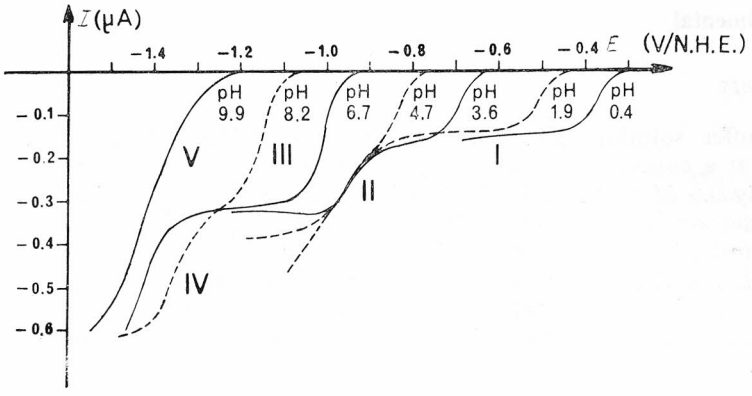


Fig. 5.
Reduction of 10^{-4} *M* aqueous solutions of pyrimidine by drop-time controlled polarography: pH 0.4 (HCl), 1.9 (phosphate), 3.6 (formate), 4.7 (acetate), 6.7 (phosphate), 8.2 (tris), 9.9 (glycine).

from the background. At about pH 5.2 waves I and II merge to form a pH-dependent wave III, whose wave current is almost twice that of wave I. At about pH 6.6, wave IV emerges from the background, and merges with wave III at about pH 10. As $E_{1/2}$ at a given pH does not shift with change of buffer nature and concentration, it may be assumed that this polarographic behavior of pyrimidine in very dilute aqueous solution does not depend on this experimental factor.

The pH-dependence of $E_{1/2}$ (Fig. 6) gives rise to the following $E_{1/2}$ *versus* pH linear relationships at this pyrimidine concentration (10^{-4} *M*):

$$\text{Wave I} \quad : E_{1/2} = [-\ 321 - 102\ \text{pH}] \pm 10\ \text{mV (N.H.E.)}$$

$$\text{Wave II} \quad : E_{1/2} = -\ 960 \pm 30\ \text{mV (N.H.E.)}$$

$$\text{Wave III} \quad : E_{1/2} = [-\ 465 - 85\ \text{pH}] \pm 15\ \text{mV (N.H.E.)}$$

$$\text{Wave IV} \quad : E_{1/2} = -1380 \pm 60\ \text{mV (N.H.E.)}$$

The pH-dependence of $-I_d/c$ (Fig. 7), for the same experimental conditions (capillary 10, 35.0 cm mercury, free drop-time 9 ± 1 s, controlled drop-time 0.5 s, m = 0.86 ± 0.03 mg s^{-1}), gives $-I_d/c$ values which do not

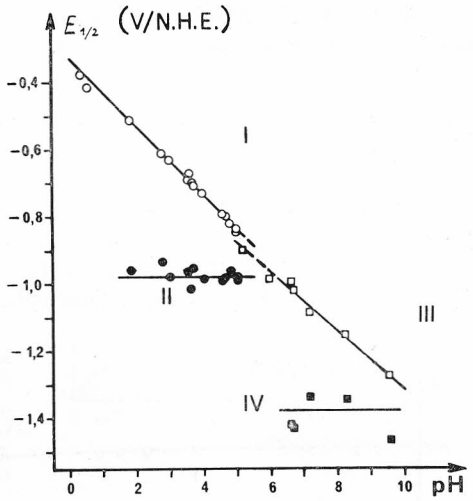


Fig. 6.
Reduction of 10^{-4} *M* aqueous solutions of pyrimidine by drop-time controlled polarography: (○) wave I, (●) wave II, (□) wave III and (■) wave IV. $E_{1/2}$ *versus* pH linear relationships.

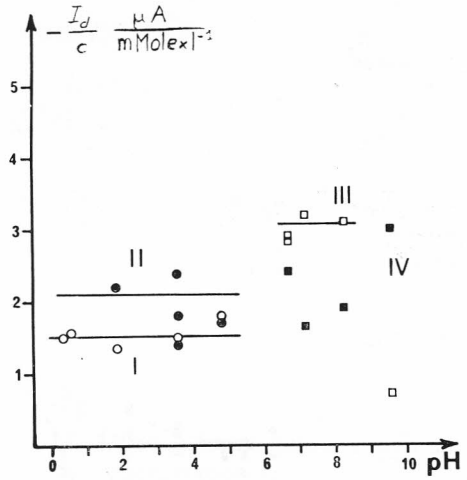


Fig. 7.
Reduction of 10^{-4} *M* aqueous solutions of pyrimidine by drop-time controlled polarography: (○) wave I, (●) wave II, (□) wave III and (■) wave IV. I_d/c *versus* pH relationships. Conditions: capillary 10, 35.0 cm of mercury, controlled drop-time 0.5 s, potential sweepra te 0.25 V min^{-1}.

vary very much from waves I and III (except the pH 9.7 value) but which can be very different for wave II and wave IV:

$$\text{Wave I} \quad : -\frac{I_d}{c} = +1.50 \pm 0.15 \ \mu \text{A/m } M$$

$$\text{Wave II} \quad : +1.45 < -\frac{I_d}{c} < 2.40 \ \mu \text{A/m } M$$

$$\text{Wave III} \quad : -\frac{I_d}{c} = 3.05 \pm 0.15 \ \mu \text{A/m } M$$

$$\text{Wave IV} \quad : +1.7 < -\frac{I_d}{c} < 3.0 \ \mu \text{A/m } M$$

As for as the number of electrons is concerned, it is worth noting that the height of wave III is almost exactly double of wave I.

Effect of pyrimidine concentration on its polarographic behavior in aqueous solution

When the pyrimidine concentration is not very low and rises above a 2 mM level the shape of the polarographic curves changes progressively and, if pH is in the 3.0-5.2 range, wave II usually splits into two different waves, II_a and II_b (see Figs. 8-12). This splitting has been observed in different

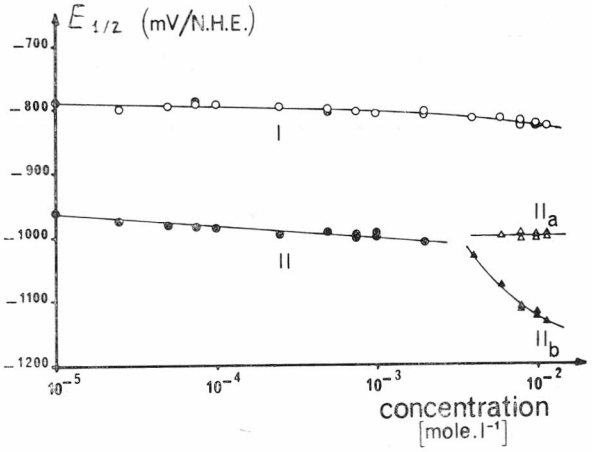


Fig. 8.
Polarographic behavior of pyrimidine in pH 4.6 acetate buffer solution: (○) wave I, (●) wave II, (△) wave II_a and (▲) wave II_b. $E_{1/2}$ *versus* pyrimidine concentration relationships.

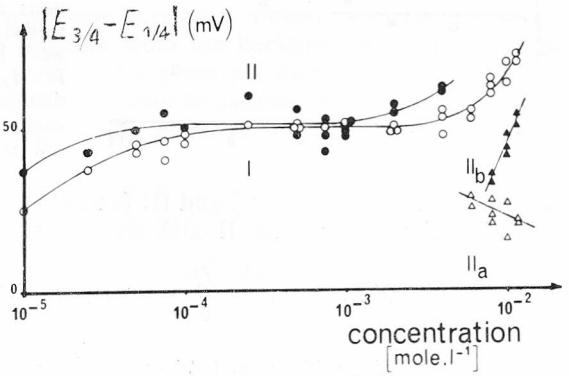


Fig. 9.
Polarographic behavior of pyrimidine in pH 4.6 acetate buffer solution: (○) wave I, (●) wave II, (△) wave II_a and (▲) wave II_b. $|E_{3/4} - E_{1/4}|$ *versus* pyrimidine concentration relationships.

buffer solutions (citrate, formate, acetate, oxalate, phthalate) and for the same pyrimidine concentration (2.3 ± 0.3 mM). As $E_{1/2}$ of the other reduction waves shift with change of pyrimidine concentration, we shall describe all the effects of this experimental factor.

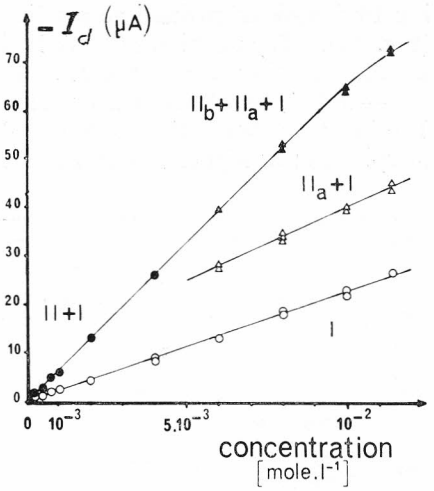


Fig. 10.
Polarographic behavior of pyrimidine in pH 4.6 acetate buffer solution: (○) wave I, (●) sum of waves I and II_a, (△) sum of waves I and II_b and (▲) sum of waves I, II_a and II_b. *I_d versu.* pyrimidine concentration relationshipss

Wave I

I_d *versus* c linear relationships are observed between pH 0.3 and 5.2 in the concentration range 10^{-5} to 10^{-2} M. $E_{1/2}$ *versus* log c linear relationships were shown in the same conditions (Fig. 11): the slope of such diagrams is usually of $+ 20 \pm 5$ mV per concentration decade at low concentration and $E_{1/2}$ is concentration independent at pH higher than 2.8 and at concentration levels above 10^{-3} M. The slope of wave I (as measured by $1/|E_{3/4} - E_{1/4}|$) is generally consistent with 1e exchange (see Fig. 9 for example), in the mean concentration range studied, *i.e.* from 10^{-4} to about 7×10^{-3} M.

Wave II

I_d *versus* c linear relationships were observed between pH 2.0 and 5.2 in the concentration range of 10^{-5} to 2×10^{-3} M. The ratio of wave II to wave I limiting currents was ranging between 1.3 and 1.7. $E_{1/2}$ *versus* log c linear relationships were observed in the same conditions (Fig. 11) : the slope, ranging between -15 and -42 mV per concentration decade, had a mean and frequent value of -35 ± 7 mV per concentration decade. In the concentration range of about 10^{-4} to about 10^{-3} M, the slope of wave II (as measured by $1/|E_{3/4} - E_{1/4}|$) is generally consistent with a 1e exchange (see Fig. 9 for example).

Wave II_a

This wave is shown when pyrimidine concentration is higher than 2.3 mM in a pH range of 3.6 to 5.2. $E_{1/2}$ and I_{lim} are generally both pH-independent (see for example Fig. 12 drawn with data obtained from solutions contain-

ing 10^{-2} mole of pyrimidine per litre) and almost concentration-independent (Fig. 11). The position of wave II_a is not very different from that of wave II at the splitting concentration. To sum up, wave II_a has a $E_{1/2}$ value of -965 ± 10 mV (N.H.E.) whatever pH and pyrimidine concentration. The height of wave II_a equals exactly that of wave II at the concentration of splitting; at higher concentrations the sum of the heights of wave II_a and

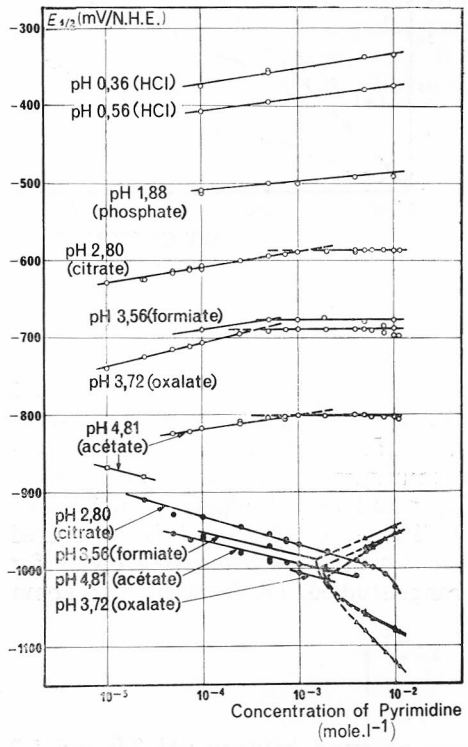


Fig. 11.
Dependence of $E_{1/2}$ on concentration of pyrimidine at different pH in aqueous solution.

wave II_b depends linearly on the pyrimidine concentration (with the same slope than wave II at lower concentrations) (Fig. 10). The slope of wave II_a as measured by $1/|E_{3/4} - E_{1/4}|$, is always high, even when waves II_a and II_b are distant enough (Fig. 9): if one assumes that wave II_a is a normal rapid wave (see later) its value would be consistent with a 2e exchange.

Wave II_b

This wave is also shown when pyrimidine concentration is above 2.3 mM in a pH range 3.6 to 5.2. In contrast with wave II_a wave II_b is very strongly concentration-dependent: $E_{1/2}$ shifts for more than 100 mV towards negative potentials when the concentration increases from 2.5 to 20 mM

(Fig. 8 and 11); I_{lim} increases in terms of concentration following the relationship:

$$\frac{1}{c}\left[(I_{lim})_{II_a} + (I_{lim})_{II_b}\right] = \frac{1}{c'}(I_d)_{II}$$

where c and c' are the concentrations of pyrimidine ($c \geq 2.3$ mM, $c' \leq 2.3$ mM) $(I_{lim})_{II_a}$, $(I_{lim})_{II_b}$ are relative to the c value and $(I_d)_{II}$ to the c' value. For

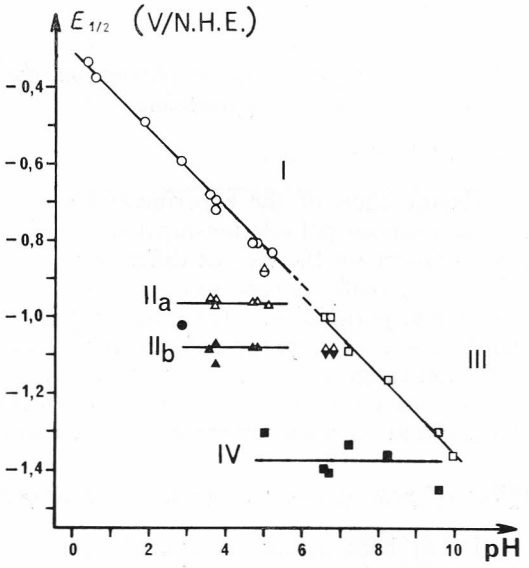


Fig. 12.
$E_{1/2}$ *versus* pH linear relationships for pyrimidine at a concentration of 10^{-2} M: (○) wave I, (●) wave II, (△) wave II_a, (▲) wave II_b, (□) wave III and (■) wave IV.

the same pyrimidine concentration *e.g.* 10^{-2} M (Fig. 12) $E_{1/2}$ is pH-independent:

$$(E_{1/2})_{II_b} = -1080 \pm 10 \text{ mV (N.H.E.)}$$

Wave III

For all the buffer solutions examined *i.e.* between pH 5.2 and 10, $E_{1/2}$ and I_d/c do not shift significantly with pyrimidine concentration in the range of 10^{-5} to 10^{-2} M. However, the $E_{1/2}$ *versus* pH relationships obtained by least-squares analysis of the experimental data seem to be somewhat different for:

$$10^{-4}\ M : E_{1/2} = [-465 - 85\ \text{pH}] \pm 15 \text{ mV (N.H.E.)}$$

and

$$10^{-2}\ M : E_{1/2} = [-335 - 102\ \text{pH}] \pm 20 \text{ mV (N.H.E.)}$$

indicating a slope change more than an actual position change in these diagrams (Figs. 6 and 12).

Wave IV

The experimental data obtained for this wave are so different from one buffer to another or from one pyrimidine concentration to another, that it is difficult to measure the characteristics of this wave accurately: $E_{1/2}$ ranges between -1330 and -1450 mV (N.H.E.) and I_d/c between -3.0 and -1.7 μ A/m M^{-1}.

Effect of the experimental conditions on the characteristics of the different reduction waves of pyrimidine

Effect of buffer solution

Examination of the experimental data used in Figs. 6, 7 and 12 ($E_{1/2}$ and $-I_d/c$) *versus* pH relationships) and in Fig. 11 (influence of the pyrimidine concentration on the $E_{1/2}$ at different pH values) indicates that there is no influence of buffer nature and concentration on the polarographic reduction behavior of pyrimidine. These data were obtained with 13 different buffers and for some of them for different buffer concentrations (acid and base concentration ranging from 3×10^{-2} to 5×10^{-1} M). The only possible exceptions in this assumption could be for our being unable to obtain close values of $-I_d/c$ and to some extent of $E_{1/2}$ for waves II an IV.

Effect of polarographic characteristics: drop-time and mercury flow

It has been already shown [19–24] that the use of a mechanically controlled drop-time does not change the shape of the I *versus* time curves and makes it possible to separate the two experimental factors of polarography: drop-time and mercury flow. To measure the characteristics of the pyrimidine reduction waves we have compared their behavior towards these two factors to the behavior of Tl^+ and Cd^{2+} reduction waves. Indeed Tl^+ and Cd^{2+} are known to give rise to rapid polarographic waves whose current is limited by diffusion. The behaviour of all the pyrimidine reduction waves *versus* mercury flow m (changing of mercury height with the same controlled drop-time) is normal: the slopes of the $\log|I_d|$ *versus* $\log m$ curves are similar to those for Tl^+ and Cd^{2+} *i.e.* $+0.85 \pm 0.07$. An example of this influence of mercury flow is given for waves I_a II_a and II_b in a solution of 10^{-2} mole of pyrimidine per litre of buffer formate (pH 3.6) in Fig. 13.

The behaviour of wave I, II, III and IV *versus* drop-time τ (changing of drop-time with the same mercury flow, *i.e.* same mercury height) is different from those of waves II_a and II_b. Whereas the first group gives rise to $\log|I_d|$ *versus* $\log \tau$ curves whose slopes are similar to those in the Tl^+ and Cd^{2+} corresponding diagrams *i.e.* $+0.25 \pm 0.06$, the second group shows negatives slopes (wave II_a) or variable abnormal slopes (wave II_b). An example of this difference is shown in Fig. 14 for pH 3.6 solutions of

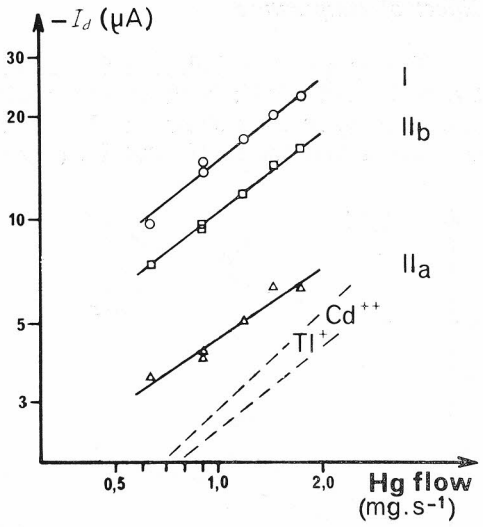


Fig. 13.
Log $|I_d|$ *versus* log (mercury flow) linear relationships for pyrimidine solutions of 10^{-2} *M* in formate buffer (pH 3.6): (○) wave I, (△) wave II_a, (□) wave II_b. Conditions: capillary 10, mercury height ranging between 25.0 and 75.0 cm, controlled drop-time 0.5 s. The mercury flows were measured with freely dropping mercury in deionized water and decrease less than 10 % if the 0.5 s drop-time is imposed.

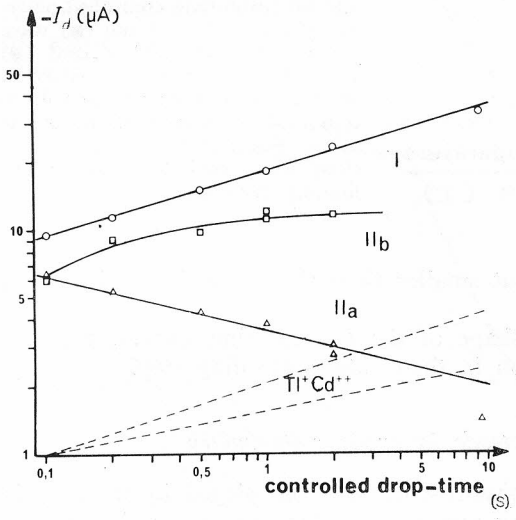


Fig. 14.
Drop-time dependence of the limiting current of the reduction waves of 10^{-2} *M* pyrimidine solutions in formate buffer (pH 3.6): (○) wave I, (△) wave II_a and (□) wave II_b. Conditions: capillary 10, mercury height 35.0 cm, free drop-time 9.2 ± 0.1 s, mercury flow with freely dropping mercury 0.86 ± 0.03 mg s^{-1}. Peaks of the current oscillations were used to determine the limiting currents.

pyrimidine at a concentration of 10^{-2} mole per litre. The negative value of the slope corresponding to wave II_a can be nearly nil with other capillaries or other mercury flows. The lowering of the limiting current when the controlled drop-time increases is generally explained by an adsorption reaction controlling the rate of the electron transfer. Here this hypothesis has to be rejected because the current *versus* time curves for waves I, II_a and II_b have all the same normal shape in conventional *d.c.* polarography and in drop-time controlled *d.c.* polarography, the slope of log $|I|$ *versus* log time curves ranging between 0.25 and 0.30.

Effect of temperature

The temperature influence was studied in acetate buffer at pH 4.8 between 10 and 40 °C: both conventional polarography and drop-time controlled polarography show no significant difference between the behavior of wave I and waves II_a and II_b (Fig. 15). The slopes of log $|I|$, *versus*

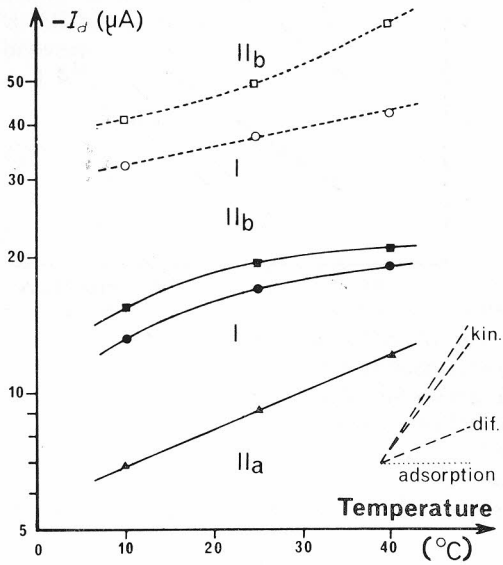


Fig. 15.
Temperature dependence of the limiting current of the reduction waves of 10^{-2} *M* pyrimidine solutions in acetate buffer (pH 4.8) both with conventional *d.c.* polarography (– – –) and with drop-time controlled polarography (——): (○) and (●) wave I, (▲) wave II_a and (□)and (■) wave II_b. Conditions: capillary 3, 70 cm mercury height, free drop-time 6.4 s, controlled drop-time 0.5 s. Peaks of the current oscillations were used to measure the limiting currents.

temperature curves are somewhat smaller than the theoretical value for a diffusion controlled wave.

As already shown by the shape of the *I versus* time curves, waves I, II, and II, involve no adsorption in their rate controlling step.

Effect of the nature of the electrode in cyclic voltammetry

In order to find out any physico-chemical role played by mercury in polarography, cyclic voltammetry was used under similar conditions with a D.M.E. (drop formation synchronized with potential sweep) and with a stationary vitreous carbon electrode. Using the same sweep rate conditions ($1\ V\ s^{-1}$) the two electrodes gave similar results between pH 2.3 and 8.2 for waves I, III and IV: the peak potentials were shifted from 130 ± 30 mV towards negative potentials when compared to the relative half-wave potentials. Instead of the two waves II_a and II_b the cyclic voltammetry with a D.M.E. gave one peak II showing a shoulder on the less negative side: this peak II is placed at 30 mV below wave II_b halfwave potential in the same concentration conditions (10^{-2} *M*). These experimental data are in good agreement with data of ELVING *et al.* (presented in Fig. 3) for car-

bon electrode but give somewhat different results for cyclic voltammetry on D.M.E.

To sum up the effects of experimental conditions on the reduction behavior of pyrimidine in aqueous solution, we have found that the nature and concentration of the buffer, and the nature of the electrode do not significantly alter the shape and position of the different waves. Temperature and mercury flow in polarography have the same, " normal " influence on the different waves described. The controlled drop-time polarography seems to be the only way to differentiate the split waves observed at higher concentrations in acidic media from the normal waves observed with very dilute solutions. Consequently the reduction behaviour of pyrimidine gives data of energetical significance even if reactions occur at somewhat limited rates.

Conclusions

The concentration of pyrimidine is a fundamental factor in the study of its reduction properties and until now has not received sufficient attention. Around a value of 10^{-3} *M* there is a change in behavior both for wave I at pH above 2.8 (Fig. 11) and for wave II (Figs. 8 to 10). This is why the results are to be discussed for concentrations above and below this value.

In very dilute solutions, *i.e.* in a concentration range of 10^{-5} to 10^{-3} *M*, the pyrimidine reduction waves all show the same behavior towards experimental factors (nature and concentration of buffer, mercury flow and drop-

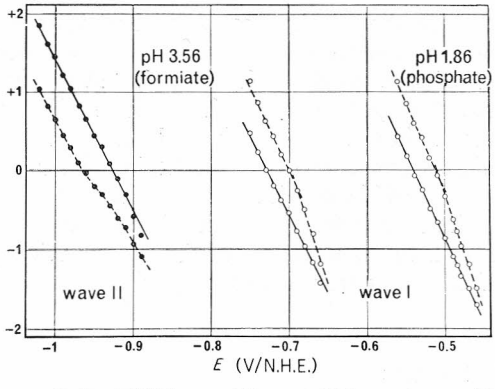
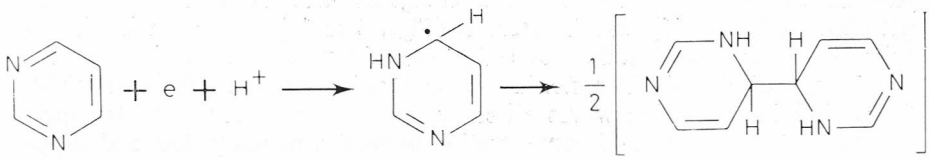


Fig. 16.
Logarithmic analysis of the polarographic reduction waves of pyrimidine (10^{-4} *M*) in aqueous buffered solutions: pH 1.86 (phosphate) and pH 3.56 (formiate).
(- - - -) conventional logarithmic analysis *i.e.* log $[I/(I_{lim} - I)]$ *versus* *E*
(——) logarithmic analysis in presence of a dimerization: log $[I^{2/3}/(I_{lim} - I)]$ *versus* *E* for (○) wave I, log $[I^{3/2}/(I_{lim} - I)]$ *versus* E for (●) wave II. Conditions: capillary 10, 35 cm mercury, controlled drop-time 0.5 s, $m = 0.86 \pm 0.03$ mg s^{-1}.

time, temperature, nature of electrode) except towards pyrimidine concentration. The half-wave potential of wave I shifts towards less negative potentials (+ 20 mV/concentration decade) whereas the half-wave potential of wave II shifts towards more negative potentials (— 35 ± 7 mV/concentration decade). This type of concentration dependence obviously means

the existence of a chemical step between the wave I and wave II reduction steps.[25-27] Similar behaviors were observed during reduction of aromatic aldehydes and ketones [28,29] and of N-alkyl pyridinium salts [30,31] and were both explained as a dimerization of the radical formed during the first step. To give proof of the mechanisms MAIRANOVSKII [27] tried a logarithmic analysis of the polarographic waves and found log $[I^{2/3}/(I_{lim} - I)]$ *versus* E linear relationships for the first wave (electron-transfer followed by dimerization) and log $[I^{2/3}/(I_{lim} - I)$ *versus* E linear relationships for the second wave (dimerization competitive with electron transfer). We tried the same way to fit the experimental data with either log $[I/(I_{lim} - I)]$ *versus* E relationships, or log $[I^{2/3}/(I_{lim} - I)]$ *versus* E relationships for wave I at pH 1.86 and 3.56 in 10^{-4} M solutions. The results in Fig. 16 are in good agreement with the second relationship (slope $-1/(48 \pm 2)$ mV^{-1}) whereas the first gives two relationships one above $E_{1/2}$ and one under $E_{1/2}$ with different slopes ($-1/28$ and $-1/40$ mV^{-1}). Similar results were obtained (Fig. 16) for wave II at pH 3.56 with either log $[I/(I_{lim} - I)]$ *versus* E or log $[I^{2/3}/(I_{lim} - I)]$ *versus* E relationships: the second relationship gives a constant slope at $-1/47$ mV^{-1} whereas the first gives two different slopes ($-1/73$ and $-1/55$ mV^{-1}). These observations may induce to suppose a dimerization of a wave I product as:



But one should also consider other types of reaction of this radical, for example on non-reduced pyrimidine or on non-reduced protonized pyrimidine. In effect a good mechanism should fit the concentration dependence of the half-wave potential together with its pH-dependence. Moreover such a mechanism should also fit the pH and concentration-dependence at higher pyrimidine concentration and explain both the change of slope of $E_{1/2}$ *versus* log c at pH larger than 2.8 and the splitting of wave II at a concentration higher than 2.3 mM. In further investigations the different mechanisms proposed have actually to be tested for the complex pH and concentration-dependence of the reduction waves of pyrimidine.

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